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Decoupled above- and belowground responses to multi-decadal nitrogen and phosphorus amendments in two tundra ecosystems

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Abstract. Global change in the Arctic promotes deeper soil thaw and enhanced soil microbial activity, increasing nitrogen (N) and phosphorus (P) availability to plants and microbes in strongly nutrient-limited ecosystems. This critical, positive climate feedback has been examined through fertilization experiments that describe short-term (<10 yr) above- or belowground responses to combined NP additions, with evidence of enhanced shrub growth, nutrient availability, and soil organic matter decomposition. There has been less opportunity for long-term comparisons of both above- and belowground responses with factorial N and P additions in different systems, despite broad awareness that ecosystem response can shift with time, and the potential for decoupled above- vs. belowground or N vs. P responses, currently and with further predicted global change. We examined the response of the plants, soil microbes, and soil nutrients, to factorial N and P additions in the moist acidic tundra (MAT; 26 yr of nutrient additions) and moist non-acidic tundra (MNT; 16 yr). Aboveground, the MAT plant community continues to change as predicted by earlier studies: Functional groups responded independently to N and P, but NDVI-biomass, especially of *Betula nana*, only increased with N addition. Unlike shorter-term MNT studies, the MNT vegetation, which does not include *B. nana*, shows few new fertilization responses. Belowground responses were not predicted by aboveground responses in either MAT or MNT. In contrast to the N response aboveground, MAT microbial biomass responded positively and microbial phosphatase activity negatively to P additions, implying possible release from microbial P limitation. Critically, earlier published results of declines in soil total carbon (C) with combined NP addition in the MAT are not present in the long term. We make two conclusions: (1) Arctic ecosystems are not universally N-limited but also exhibit complex responses to P alone or in combination with N; and (2) the presence or absence of key vegetation species can cascade from aboveground to belowground and restrict the extrapolation of responses of nutrient addition in a single arctic ecosystem to other arctic ecosystems, the short-term to the long term, or aboveground to belowground.

Key words: extracellular enzyme activity; fertilization; long term; microbial biomass; moist non-acidic tundra; nutrient limitation; soil carbon; Toolik LTER.

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INTRODUCTION

Arctic regions are warming more rapidly than other parts of the globe (Huang et al. 2017) resulting in rapid ecological changes in arctic

ecosystems (Post et al. 2009). Organisms respond both directly to warmer temperatures, but also to changes in ecosystem properties resulting from the warming. For example, deeper soil thaw is expected to enhance nutrient availability in the

Arctic through increased rates of decomposition and nutrient mineralization (Nadelhoffer et al. 1991, Hobbie et al. 1999, Hinzman et al. 2005, Wang et al. 2017). Interest in how plant and soil communities will respond to increases in nutrient availability has resulted in a number of long-term fertilization experiments in a variety of arctic ecosystems across North America (Shaver et al. 2014, Gough et al. 2016) and Scandinavia (Jonasson et al. 1999, Van Wijk et al. 2004). Although the long-term levels of nutrient additions are often unrealistic in terms of nutrient release with warming, these experiments have been extremely fruitful for characterizing responses to changing ecosystem nutrient limitations, and the effects of fertilization aboveground (Chapin et al. 1995, Bret-Harte et al. 2001, Campioli et al. 2012), belowground (Jonasson et al. 1999, Rinnan et al. 2007, Deslippe et al. 2011, Koyama et al. 2013), and to a limited extent, above- and belowground combined (Chapin et al. 1995, Gough et al. 2002, Mack et al. 2004, Rinnan et al. 2007, Haugwitz et al. 2011), are well described. Resampling of these experiments, however, has highlighted the importance of time, as the longer-term responses rarely reflect responses of the shorter term (<10 yr). For instance, responses of particular plant groups to nutrient additions may be positive in the short term but negative in the longer term (Campioli et al. 2012, Shaver et al. 2014), and the soil microbial community may require more than 10 yr of fertilization to show effects (Rinnan et al. 2007). Long-term fertilization experiments that more extensively explore above- and belowground responses are especially useful for helping us understand the whole-ecosystem response to changes in nutrient availability.

The type of nutrient addition also limits our knowledge of tundra responses to long-term increased nutrient availability: Historically, researchers have favored combined N and P (NP) fertilization responses, whereas the independent (factorial) addition of N and P is less common. NP fertilization experiments (control and NP combined treatments only) have provided good evidence in arctic ecosystems that nutrient addition alters vegetation structure (e.g., increasing dominance by deciduous shrubs [Shaver et al. 2001, Mack et al. 2004], enhances soil nutrient availability [Chapin et al. 1995], and

enhances soil organic matter [SOM] decomposition [Mack et al. 2004, Koyama et al. 2013]). However, soil N and P cycles are not always coupled (Rastetter et al. 2013, Sundqvist et al. 2014) and may decouple further with global change. For instance, arctic mineral soils are often frozen, such that the short-term source of P for most tundra plants and microbes may be via recycled organic matter P (Chapin et al. 1978, Giblin et al. 1991, Jonasson et al. 1999), and thus, N and P may respond differently to enhanced soil thaw over longer time periods. In addition, early experiments in Alaskan ecosystems that examined vegetation responses to factorial N and P additions found a consistent increase in growth with N addition (albeit with a strong interaction between N and P; Shaver and Chapin 1980), and faster accumulation of N than P in tussock sedges when both nutrients were added (Shaver and Chapin 1986, 1995), promoting the paradigm that arctic ecosystems are strongly N-limited. However, theoretical P limitation or NP co-limitation proposed by these same authors lead to the initiation of Alaskan tundra factorial N and P experiments. This theory has been supported in short-term N and P factorial fertilization experiments in Alaskan, Canadian, and Swedish tundra ecosystems that report potential N and P co-limitation of numerous arctic ecosystems (e.g., heath tundra, Gordon et al. 2001, Gough et al. 2002, Sundqvist et al. 2014, Street et al. 2018; mesic meadow, Giesler et al. 2012; birch hummock, Zamin and Grogan 2012), as well as evidence that some carbon (C) rich ecosystems, such as wet sedge tundra, may be primarily P-limited (Chapin et al. 1975, Shaver and Chapin 1995, Nadelhoffer et al. 2002). Therefore, an assessment of the long-term effects of factorial N and P addition on both above- and belowground properties is necessary for understanding potential ecosystem responses to shifting nutrient supplies with a changing climate, even in systems that are historically described as N-limited.

The objectives of this study were fourfold: (1) to characterize the long-term response of nutrient amendments (N, P, and NP) to arctic tundra in comparison to earlier studies; (2) to investigate similarities in response above- and belowground; (3) to compare the response in two common arctic tundra ecosystems; and (4) to understand whether these systems are responsive to P in

addition to N. We examined these objectives using multi-decadal, factorial N and P fertilization experiments in both moist acidic tundra (MAT) and the geologically younger moist non-acidic tundra (MNT) at Toolik LTER, Alaska. MAT is the most common arctic vegetation type in northern Alaska, and MNT is the second most abundant vegetation type in the region (Raynolds et al. 2006). At these sites, we measured the vegetation community structure, aboveground biomass based on normalized difference vegetation index (NDVI), soil nutrient stocks and extractable pools, the microbial biomass, and the activity of three extracellular enzymes that are representative of organic matter decomposition. Although there have been several studies that have examined aspects of these control and NP plots, the effects of the full-factorial fertilization experiment on plant and soil properties have not been published for the 26-yr history of the experiment (MAT), or since year 4 in the experiment's 16-yr duration (MNT). In response to our objectives, are hypotheses are also fourfold: (1) Long-term responses will differ from earlier published NP results, as feedbacks between above- and belowground adjust over time; (2) above- and belowground responses will be decoupled, in response to the competition between plants and soil microbes; (3) there will be ecosystem-specific responses that reflect the unique plant and microbial communities; and (4) we will find responses to N and P alone and interactively, reflecting the potential for arctic ecosystems, or components of arctic ecosystems, to be limited by both nutrients.

MATERIALS AND METHODS

Study site

The study was conducted at the Toolik LTER in the northern foothills of the Brooks Range, Alaska (68°38'N and 149°43'W, elevation 760 m). Annual average temperature is -7°C , and temperatures are generally above freezing between June and August, with an average temperature of 10°C in July (Shaver et al. 2006). The area is underlain by continuous permafrost ~ 200 m thick, so there is no deep drainage of soil water and little or no connection with groundwater (Hinzman et al. 1991), with the depth of the active layer ranging from 30 to 50 cm and they

may not thaw each year to the mineral layer. Maximum thaw occurs in late August to early September. Soils are gelisols (formed over permafrost), with high organic content (Shaver et al. 2014).

We conducted this study in two contrasting tussock tundra ecosystems: MAT and MNT (Table 1). Tussock tundra is the most widespread vegetation type in Alaskan upland tundra—the two ecosystems described here differ based on acidity, and correspondingly, on vegetation composition. The MAT site is located on the older Itkillik II glacial surface where the substrate is 50–120 kyr old, whereas the MNT is located on the younger Itkillik I glacial surface where the substrate is 11.5–25 kyr old (Hamilton 2003). MAT soils have a pH = 3–4, whereas the MNT soil pH is neutral (pH = 6–7). Characteristics of the soils from the two ecosystems, including bulk density and depth of the organic layer, as well as bulk nutrient concentrations, are detailed in Table 1. The two ecosystems have a similar plant functional type composition but plant biomass in the MNT is less than the MAT, and species diversity higher (Gough et al. 2000).

Experimental design

Fertilization treatments in both experiments are maintained by the Toolik LTER with a full-factorial addition of N ($10 \text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ as NH_4NO_3) and P ($5 \text{ g P}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ as P_2O_5), annually in pellet form following snowmelt (early June). At the time of our study, the MAT site had been fertilized annually for 26 yr (established in 1989) and the MNT for 16 yr (established in 1997). This study used a 5×20 m plot from each factorial N and P treatment, from three (MNT) or four (MAT) replicate blocks that were separated by a minimum of 20 m.

Vegetation sampling

Aerial percent cover was estimated in each plot in mid-July 2013. The percent cover of mosses, lichens, and all vascular plant species was visually estimated within eight $\sim 1 \text{ m}^2$ adjacent quadrats, each with 20×20 cm sub-quadrats, placed along the edge of each plot (0.25 m inside to avoid edge effects).

NDVI was used as a measure of the abundance of photosynthetically active vegetation (Rouse et al. 1974) and is highly sensitive to variation in

Table 1. Characteristics of the two arctic ecosystems in this study; mean (SE).

| Characteristic | MAT | | | MNT | | |
|-----------------------------------|------------------|-----------------|-------------|------------------|-----------------|-------------|
| | Organic (0–5 cm) | Organic (>5 cm) | Mineral | Organic (0–5 cm) | Organic (>5 cm) | Mineral |
| pH | 4.3 (0.1) | 4.3 (0.1) | 4.5 (0.1) | 7.0 (0.1) | 7.0 (0.1) | 6.9 (0.2) |
| Depth of organic | 12.0 (1.2) | 12.0 (1.2) | – | 15.7 (2.2) | 15.7 (2.2) | – |
| Bulk density (g/cm ³) | 0.10 (0.03) | 0.13 (0.01) | 0.78 (0.30) | 0.19 (0.07) | 0.37 (0.23) | – |
| Soil moisture (%) | 686.3 (104.4) | 595.0 (51.0) | 78.9 (17.4) | 426.0 (49.6) | 355.7 (19.3) | – |
| %C | 43.45 (0.46) | 39.19 (1.86) | 9.2 (3.48) | 39.75 (1.56) | 35.50 (1.69) | – |
| %N | 1.24 (0.07) | 1.22 (0.06) | 0.46 (0.18) | 1.59 (0.28) | 1.89 (0.22) | – |
| %P | 0.08 (0.01) | 0.09 (0.01) | 0.05 (0.02) | 0.09 (0.01) | 0.09 (0.01) | – |
| %Ca | 0.31 (0.06) | 0.31 (0.06) | 0.05 (0.01) | 2.53 (0.18) | 2.53 (0.18) | 0.42 (0.04) |
| %Mg | 0.12 (0.01) | 0.12 (0.01) | 0.19 (0.02) | 0.17 (0.02) | 0.17 (0.02) | 0.33 (0.01) |
| %K | 0.16 (0.05) | 0.16 (0.05) | 0.17 (0.03) | 0.11 (0.02) | 0.11 (0.02) | 0.15 (0.02) |
| %Al | 0.88 (0.22) | 0.88 (0.22) | 1.48 (0.08) | 0.66 (0.10) | 0.66 (0.10) | 1.41 (0.10) |

Notes: Moist acidic tundra (MAT) was most recently glaciated 50,000–100,000 yr ago and moist non-acidic tundra (MNT) 11,500–25,000 yr ago (Hamilton 2003). Experimental fertilizer additions were at the rate of 10 g N·m⁻²·yr⁻¹; 5 g P·m⁻²·yr⁻¹ in both ecosystems, starting in 1989 (MAT) or 1997 (MNT). % Ca, Mg, K, and Al are from Hobbie and Gough 2002, %C, N, and P measured in the control plots during this study, and pH from LTER measurements in 2013. En dash indicates data were not applicable (Depth of organic) or available.

aboveground biomass of vegetation in the MAT at this site (Boelman et al. 2005); hereafter, we refer to NDVI estimates as NDVI-biomass. We calculated the normalized difference vegetation index (NDVI) as $(\text{NIR}-\text{R})/(\text{NIR} + \text{R})$, where NIR indicates mean reflectance at near-infrared wavelengths (841–876 nm) and R mean reflectance at visible red wavelengths (620–670 nm). Spectral radiance measurements were collected by the Toolik LTER using a hand-held dual channel spectrophotometer (Unispec DC, PP Systems, Amesbury, Massachusetts, USA; Shaver and Gough 2015). Radiance measurements were taken throughout the summer of 2014 on multiple dates for each plot (2013 measurements were not available for both ecosystems). On each date, five replicate scans were taken 1 m apart along a 5 m transect located ~0.5 m from the edge of each plot. For this study, we selected the dates that occurred nearest to peak NDVI measurements (10 July, 28 July, 6 August, and 9 August). All spectral measurements were converted to reflectance values and were interpolated to 1-nm intervals. Vegetation indices were calculated for replicated scans and then averaged per block and across all four collection days before analysis.

Vegetation data were analyzed as relative percent cover for each species and for functional groups (calculated as the sum of all component species). Relative percent cover was calculated by dividing the cover of the individual species or

functional group in each plot by the total plant cover recorded for that 1-m² plot.

Soil sampling and preparation

Organic and mineral soil horizons were sampled from MAT and MNT in early July 2013. When present, tussocks of *Eriophorum vaginatum* were avoided when sampling so sampling occurred only in intertussock areas (moss dominated areas between tussocks), because of distinct microtopographic differences between tussock and intertussock areas. In the MAT, *E. vaginatum* grows in dense tussocks which cover ~20% of the ground surface area, whereas the remainder of the surface area, and dominant ground cover type, is classified as intertussock and is composed of moss that is well colonized by evergreen and deciduous shrubs at both sites. In the MNT, both *E. vaginatum* and tussocks are less abundant, and tussocks cover <10% of the ground surface area. A single ~10 × 10 cm column of soil was cut from each plot to the depth of the permafrost using a serrated knife. All organic horizons were <20 cm deep and were separated into the upper organic (0–5 cm depth) and lower organic (5–15 cm depth) layers, to allow comparison with previous studies that separated by depth (Mack et al. 2004, Sistla and Schimel 2013) and because ecosystem nutrient pools and microbial biomass can vary strongly by depth in the organic horizon. The mineral layer was sampled either to permafrost, or the

upper 10 cm only, whichever was less. Mineral soils were sampled for MAT only, as the permafrost extended into the organic horizon for all MNT plots, preventing mineral soil sampling.

Soils were separated into layers in the field, bulk density subsamples were collected using a sharp knife from the center of each layer and measured, and then all samples were returned to the field laboratory. Bulk density samples were weighed and oven-dried for calculating gravimetric water content and CN analysis. The rest of the samples were homogenized by hand and all large roots (>1 mm diameter) removed. The homogenized soil was frozen at -20°C and shipped to University of California Santa Barbara for the analyses below.

Soil and microbial extraction and analysis

Soil samples (10 g) were extracted from frozen with 40 mL of deionized water by shaking for 3 h. Duplicate samples for estimates of the microbial biomass flush (fumigated) were extracted in the same manner but with the addition of 1 mL CHCl_3 (Fierer et al. 2003). All extracts were vacuum filtered through 1- μm pore size glass fiber filter paper and sparged for 30 min with compressed air (to remove residual C from the CHCl_3), then frozen at -20°C until analysis.

Water-extractable organic C (EOC) and total N (ETN) contents in the fumigated and non-fumigated extracts were determined by oxidative combustion and infrared (EOC; Nelson and Sommers 1982) or chemiluminescence (ETN) analysis (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan). Water-extractable $\text{NH}_4^+\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$ in non-fumigated extracts and $\text{PO}_4\text{-P}$ in the fumigated extracts were determined colorimetrically, using automated flow analysis (Lachat autoanalyzer) and the salicylate ($\text{NH}_4^+\text{-N}$), sulfanilamide ($\text{NO}_3\text{-N}$) and molybdate blue ($\text{PO}_4\text{-P}$) methods (Mulvaney 1996).

Microbial biomass C, N, and P flushes were calculated as the difference between EOC, ETN, or $\text{PO}_4\text{-P}$ in fumigated and non-fumigated extracts. No correction factor was applied for incomplete CHCl_3 -release, or sorption of P because these values are not known for water extraction for these two ecosystems. The final nutrient concentration of all extracts was corrected for dilution by the water content of the sample.

Soil microbial extracellular enzyme analysis

We assayed for the activity of three hydrolytic enzymes that acquire carbon, nitrogen, and phosphorous at the terminal stages of organic matter decomposition: cellulose-degrading β -glucosidase, chitin-degrading N-acetyl-glucosaminidase (NAG), and phosphatase (McLaren et al. 2017). Soil was thawed and blended with 0.05M acetate buffer (MAT: pH 5; MNT: pH 6). Soil slurries were pipetted onto 96-well plates to which fluorescing 4-methylumbelliferone (MUB) tagged substrate (β -D-glucoside, N-acetyl- α -D-glucosaminide and phosphate) was added, with 8 analytical replicates per soil. The assays were incubated at 22°C for $\sim 2\text{--}4.5$ h (previously determined, by substrate, for these soils, to be during the phase of linear increase in activity), and then, the reaction was stopped by adding 20 μL of 0.5 M NaOH. Sample fluorescence was read with a TECAN Infinite Pro 200 plate reader (Tecan Group Ltd., Männedorf, Switzerland) at 365 nm excitation, 450 nm emission. For each substrate, we measured the background fluorescence of soils and substrate and the quenching of MUB by soils and used standard curves of MUB to calculate nmol of substrate hydrolyzed per hour per g of soil. Soil pools and rates were converted from $\mu\text{g/g}$ to g/m^2 and $\text{nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ to $\text{mmol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ by correcting for the depth and bulk density of each replicate sample.

Total soil CNP analysis

Soil CN was analyzed on dried, ground soil for each soil layer using a dry combustion total CN analyzer (Perkin Elmer 2400 at NC State University Environmental Testing Service). Soil total P was analyzed using a strong-acid soluble digest (EPA method 3050B digested at NC State University Environmental and Agricultural Testing Service Laboratory). The digestates were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Model 8000, Waltham, Massachusetts, USA) with a cross-flow nebulizer.

Statistical models

We analyzed for differences in vegetation functional groups between ecosystems and treatments using a fully factorial MANOVA with site, N and P as the main factors. As the MANOVA for functional group was significant (Roy's Max

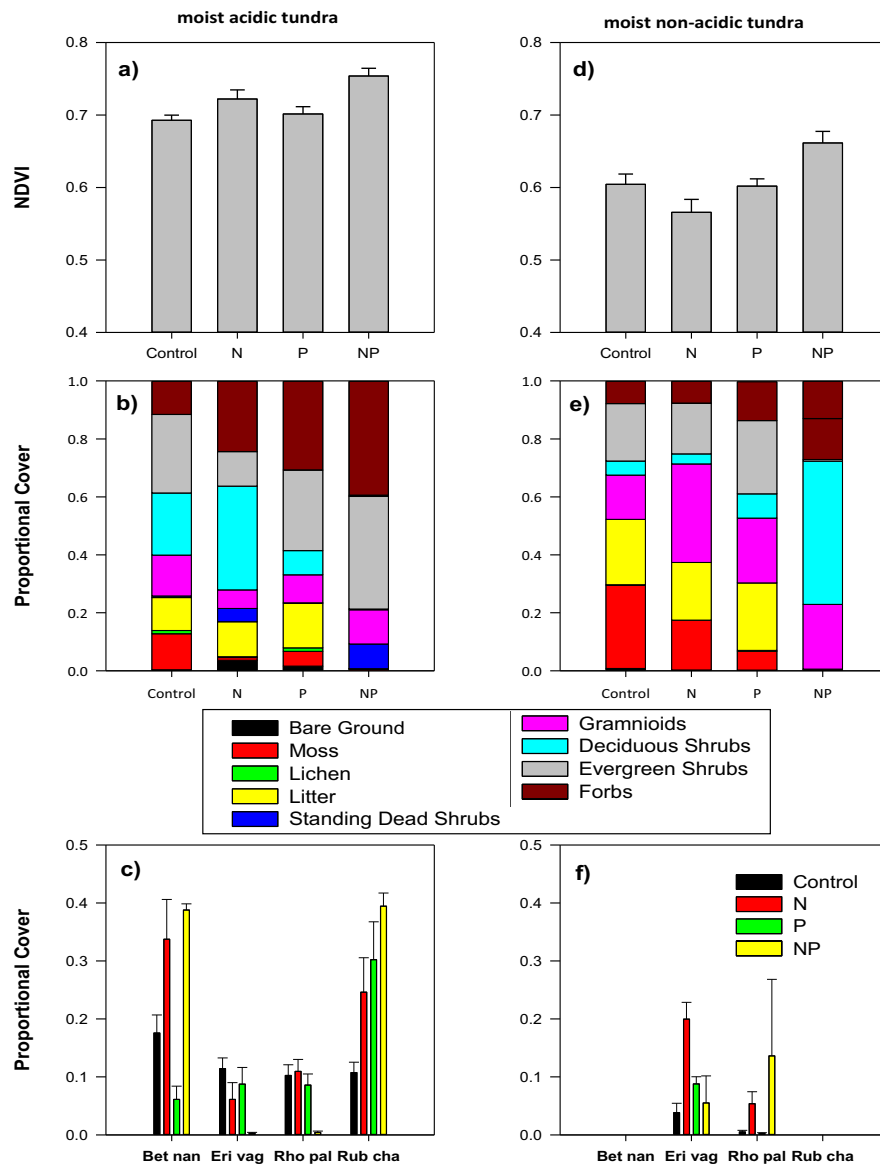


Fig. 1. The effects of long-term nutrient additions on normalized difference vegetation index (NDVI) (a,d), proportional cover of vegetation functional groups (b,e), and dominant species (c,f), in moist acidic tundra (MAT: a,b) and moist non-acidic tundra (MNT: c,d). The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Functional groups in (b) and (e) are represented by color as indicated in the legend and also are arranged top to bottom as in the legend. Abbreviations are Bet nan, *Betula nana*; Eri vag, *Eriophorum vaginatum*; Rho pal, *Rhododendron palustre*; Rub cha, *Rubus chamaemorus*.

Root $P < 0.05$), and includes significant interactions between sites and nutrient additions, each functional group was tested independently for each site using a fully factorial mixed model with

N and P as the main factors and block as a random effect (REML), for each ecosystem. As the dominant species were different between sites, we did not run the full ecosystem and treatment

model on species-specific cover. Instead, dominant species at each site (>5% cover) were each analyzed independently using a fully factorial model as above. Our models for NDVI, soil pools, and rates were similar, with block as a random effect, and N, P, and depth (the latter not for NDVI) as fixed effects, for each ecosystem. The factors N and P were treated as binary dummy variables, such that Control plots = (0, 0), N addition plots = (1, 0), P addition plots = (0, 1), and N + P addition plots = (1, 1), for the factors (N, P), respectively. For MAT soil, we analyzed the organic and mineral soil separately, in order to allow a qualitative comparison between ecosystems (mineral soil results are presented in Appendix S2: Tables S1 and S2). Because of the different time since experimental inception, we did not include both ecosystems in the same model, although we do discuss qualitative differences between the two systems. To enable this comparison, all data are presented on a per m² basis (scaled by depth and bulk density, see Table 1). Results on a per gram basis are available in the online data archive (see Data Accessibility Statement). All data were assessed for normality (Shapiro–Wilks) and heteroscedasticity and in most cases log-transformed (or square root arcsine transformed for vegetation data) before analysis with JMP 10.0 (2010, Cary, North Carolina, USA).

RESULTS

Vegetation responses to long-term nutrient addition

Overall, the vegetation responses to both N and P additions were stronger in the MAT than the MNT (Fig. 1, Table 2; Appendix S1: Tables S1 and S2). In the MAT, NDVI-biomass increased with N addition, but did not respond to P (Fig. 1; Appendix S1: Table S1). Two functional groups responded interactively to N × P: The decrease in evergreen shrubs with N was moderated by P, and the increase in deciduous shrubs with N overwhelmed the generally negative effect of P. Moss and graminoids decreased with the addition of N, and graminoids also decreased with the addition of P. Forbs increased with the addition of N and P, independently. The MAT response was dominated by *Betula nana* (deciduous shrub) and *Rubus chamaemorus* (forb), two

species that were in low abundance in the MNT (Fig. 1c, f; Appendix S1: Table S2).

In the MNT, NDVI-biomass did not change with either N or P additions, and there was no interactive response. Graminoids responded positively to N, and moss responded negatively to P. There were no N × P interactive responses in the MNT.

Soil microbial biomass C, N, and P responses to long-term nutrient addition

In the MAT organic soils, both N and P additions, as well as depth, altered microbial biomass (Table 2; Appendix S1: Table S3). MBC in MAT shallow organic soils decreased with P addition but increased with P addition in deeper organic soils (P × Depth) and did not respond to N at either depth (Fig. 2a). MAT MBN decreased with N addition, especially in deeper organic soils (N × Depth; Fig. 2b). Similar to MBC, MAT MBN decreased with P addition in shallow organic soils, but increased in deeper organic soils (P × Depth; Fig. 2b). MAT MBP increased with P addition, but only in deeper organic soils (P × Depth; Fig. 2c).

Control plot MBC in the MNT was double that in the MAT (Fig. 2a, d), and there were fewer effects of N and P addition on MBC in the MNT compared with the MAT. In the MNT, MBC and MBN did not change with N or P addition (Table 2, Fig. 2d, e; Appendix S1: Table S4), whereas MBP increased with P addition but only in deeper organic soils (Table 2, Fig. 2f, P × Depth; Appendix S1: Table S4).

Potential extracellular enzyme activity responses to long-term nutrient addition

In the MAT organic soils, there was no effect of any fertilization treatment, or depth, on the activity of β-glucosidase (Table 2, Fig. 3a, Appendix S1: Table S3). In contrast, the activity of NAG showed an N × P interaction, where the decrease with N addition was moderated by the simultaneous addition of P (Fig. 3b). Similarly, the N × P interaction for phosphatase activity in the MAT organic soils was driven by a decrease with the addition of P, and this decrease was moderated when N was added simultaneously (N × P; Fig. 3c).

Control plot β-glucosidase and NAG activity was ~10 times larger in MNT than in MAT organic soils, although control plot phosphatase activity was a similar magnitude in the two

systems (Fig. 3). Unlike the generally negative effect of fertilizers on enzyme activity in the MAT, the effect of nutrient additions on MNT enzyme activity was positive (Table 2; Appendix S1: Table S4). The activity of β -glucosidase increased with the addition of N and with organic soil depth (Fig. 3d). NAG activity in the MNT also increased with N, more so with P addition ($N \times P$; Fig. 3e). Phosphatase activity in the MNT increased with N addition (Fig. 3f).

Soil Extractable C, N, and P responses to long-term nutrient addition

Extractable organic carbon in the MAT organic soils did not respond to fertilization (Table 2, Fig. 4a; Appendix S1: Table S3), and extractable total nitrogen increased with N addition (Fig. 4b). The two inorganic components of total N (ammonium and nitrate) responded to both N and P addition in MAT organic soils: Extractable NH_4^+ -N increased with N addition, and independently decreased with P addition (Fig. 5a). Similarly, extractable NO_3^- -N increased with N addition, but this increase was moderated by the simultaneous addition of P ($N \times P$; Fig. 5b). Effects of N or P additions on soil phosphate in the MAT organic soils were complex with interactions between $N \times P \times \text{Depth}$: Extractable PO_4 -P increased with P addition, an effect that was decreased with the simultaneous addition of N ($N \times P$), especially in deeper organic soils ($N \times P \times \text{Depth}$; Fig. 4c).

MNT control plot extractable total C and N pools (ETN and NO_3^- -N) were generally similar between MAT and MNT (Table 2, Fig. 4; Appendix S1: Table S4), although control plot extractable PO_4 -P and NH_4^+ -N in MNT were \sim double of that in the MAT (Fig. 4c, f). Extractable organic C in the MNT increased with depth (Fig. 4d). Total extractable N in the MNT increased with N addition (Fig. 4e). Extractable NO_3^- -N in the MNT increased with N addition (Fig. 5d), and extractable phosphate increased with P addition (Fig. 4f).

Soil total C, N, and P responses to long-term nutrient addition

In MAT organic soils, soil total C and total soil N both increased with depth but did not respond to either N or P addition (Table 2, Fig. 6a, b; Appendix S1: Table S3). Soil total P increased with

both P addition and with depth (Fig. 6c). As with other MNT control plot variables, total soil pools of C, N, and P in the MNT were \sim double the MAT (Fig. 6). Soil total C in the MNT increased at depth but did not respond to fertilizer (Table 2, Fig. 6d; Appendix S1: Table S4). Soil total N in the MNT was higher in the deeper organic soils and increased when N and P were added together ($N \times P$; Fig. 6e). Soil total P in the MNT increased when both nutrients were added in combination ($N \times P$), and this interaction was enhanced in the surface organic soils ($N \times P \times \text{Depth}$; Fig. 6f).

DISCUSSION

We had a four-part objective in this study: (1) to characterize the long-term response of factorial nutrient (N and P) amendments to arctic tundra in comparison to earlier studies; (2) to investigate similarities in response above- and belowground; (3) to compare the response in two common arctic tundra ecosystems; and (4) to understand whether these systems are responsive to P in addition to N. The two ecosystems that we studied differed in vegetation, soil, and microbial response to similar long-term N and P additions, with predictably complex interactions. Nonetheless, in both ecosystems we found that (1) the early, strong ecosystem response to NP additions above- and belowground has been maintained, although in support of our first hypothesis, the components of these responses have changed through time. (2) In this first study to examine a complementary suite of above- and belowground responses to factorial nutrient additions in Alaskan tundra, our second hypothesis was supported, and we found a decoupling of the above- and belowground responses, highlighting that aboveground responses cannot necessarily be used to predict those belowground. (3) Our third hypothesis regarding ecosystem-specific responses was also supported in that we found a higher number of responses aboveground in the MAT as compared to the MNT and many more interactive belowground responses in the MNT and thus conclude that arctic tundra ecosystems have different sensitivities to long-term nutrient amendments. Finally, (4) our fourth hypothesis about N and P responses was also supported as we found multiple indications of ecosystem response to P, in addition to N and their

Table 2. Effects of long-term N and P addition on NDVI-biomass and abundance, soil microbial and nutrient pools, and process rates in moist acidic tundra (MAT, experiment established 1989) and moist non-acidic tundra (MNT, experiment established 1997).

| Response category | Response variable | MAT | | | MNT | | |
|--|---------------------------------|------------|------------|--------------------|------------|------------|--------------------|
| | | N response | P response | Net N × P response | N response | P response | Net N × P response |
| Vegetation biomass | NDVI | + | | | | | |
| Vegetation functional group relative abundance | Moss | – | | | | – | |
| | Lichen | | | | | | |
| | Evergreen | – | + | – | | | |
| | Forb | + | + | | | | |
| | Graminoid | – | – | | + | | |
| Microbial biomass pools | Deciduous | + | – | + | | | |
| | MBC | | +D | | | | |
| | MBN | –D | +D | | | | |
| | MBP | | +D | | | +D | |
| Extracellular enzyme rates | BG | | | | + | | |
| | NAG | | | + | – | | + |
| | Phosphatase | | – | + | + | | |
| Soil extractable pools | Organic C | | | | | | |
| | Total N | + | | | + | | |
| | NH ₄ ⁺ –N | + | – | | | | |
| | NO ₃ [–] –N | + | – | – | + | | |
| | PO ₄ –P | | +D | –D | | + | |
| Soil total pools | C | | | | | | |
| | N | | | | + | | + |
| | P | | + | | + | + | +D |

Notes: Summary data for each ecosystem are based on a two-way factorial ANOVA (N × P) for vegetation responses and a three-way ANOVA (N × P × Depth) for soil responses. Cells are filled for significant ($\alpha = 0.05$) individual responses to N or P addition or N × P interactions (light gray = positive or dark gray = negative), and D an interaction between N, P, or N × P and soil depth. Non-significant responses are not displayed. Two- and three-way interactions have complex results that are described in more detail in the text and figures. In general, a positive (light gray) interaction indicates that one factor enhanced the response of another, whereas a negative (dark gray) interaction indicates that one factor inhibits another. + is positive effect; – is negative effect, and D is N or P interaction with depth.

interactions, in vegetation and in soil, implying a need to expand our investigation of P in tundra ecosystems. We expand upon these phenomena below.

Aboveground community and NDVI-biomass response to multi-decadal nutrient amendments

There were strong responses of P addition on the cover of multiple vegetation functional groups in the MAT. We found decreases in the proportion of graminoids and increases in forbs with P additions. The response of these functional groups mirrors their response to nitrogen addition, indicating an independent co-limitation (i.e., they responded independently to N and P with no interaction between the two nutrients). In contrast, NDVI-biomass increased only with N, rather than P addition. This indicates that either both overall productivity and vegetation

composition are controlled by different nutrients, or that a few functional groups, which are regulated primarily by N, determine NDVI-biomass. We suggest the latter explanation given the strong response of deciduous shrubs, and *Betula nana* in particular, to fertilization treatments.

Although *B. nana* responded interactively to N and P addition (Appendix S1: Table S1), the overall response was dominated by the effect of N; when N and P were added simultaneously the plots resembled an exaggerated version of the N-alone plots with almost complete dominance of deciduous shrubs. Increases in *B. nana* with NP fertilization in this ecosystem have been attributed to high plasticity of *B. nana* growth, with *B. nana* changing biomass allocation and increasing the rate of new meristem production with fertilization (Bret-Harte et al. 2001). In contrast to our findings that N determines the

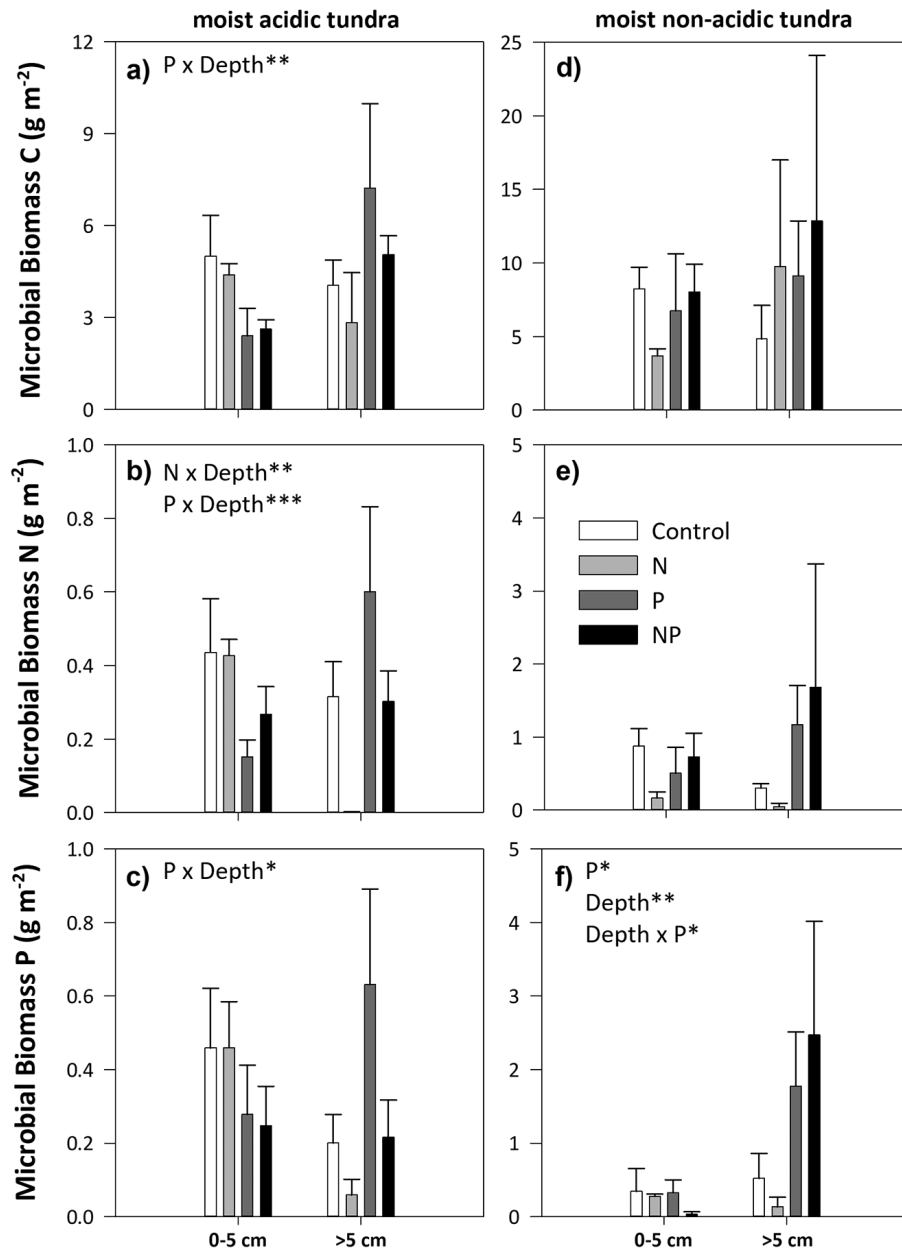


Fig. 2. The effects of long-term nutrient additions on soil microbial biomass carbon (MBC; a,d), nitrogen (MBN; b,e), and phosphate (MBP; c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d, e,f), each in the shallow (0–5 cm) and deep organic (>5 cm) soil. The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. The microbial biomass is the uncorrected flush of nutrients released upon extraction with water and chloroform. Refer to text for further experimental and method details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Asterisks next to text in plots represent significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA ($N \times P \times \text{Depth}$) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). *** $P = <0.001$, ** $P = <0.01$, * $P = <0.05$.

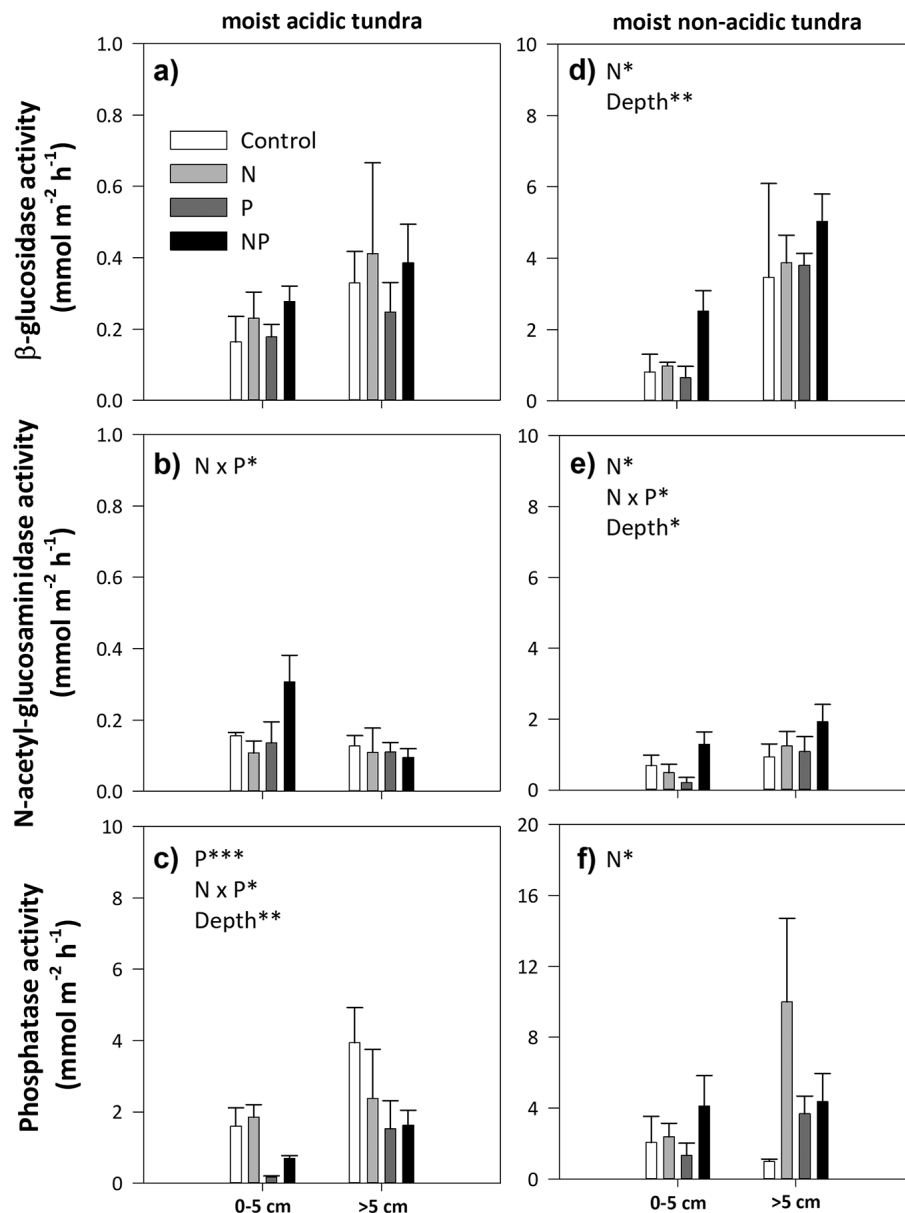


Fig. 3. The effects of long-term nutrient additions on the potential soil enzyme activity of β -glucosidase (a,d), N-acetyl-glucosaminidase (b,e), and phosphatase (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0–5 cm) and deep organic (>5 cm) soil. The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Asterisks next to text in plots represent significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA ($N \times P \times \text{Depth}$) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

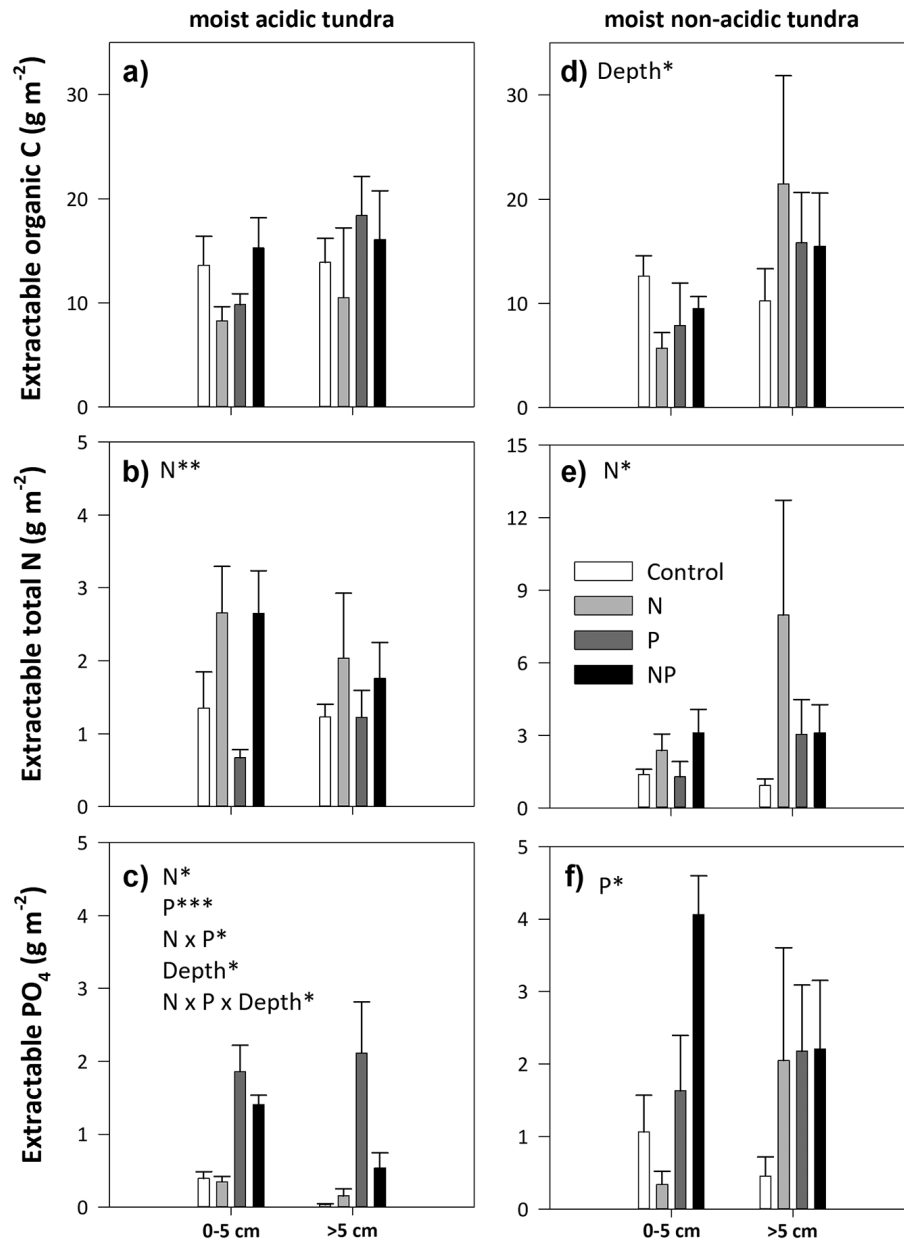


Fig. 4. The effects of long-term nutrient additions on water-extractable soil organic carbon (a,d), total nitrogen (b,e), and phosphate (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0–5 cm) and deep organic (>5 cm) soil. The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Asterisks next to text in plots represent significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA ($\text{N} \times \text{P} \times \text{Depth}$) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). *** $P = <0.001$, ** $P = <0.01$, * $P = <0.05$.

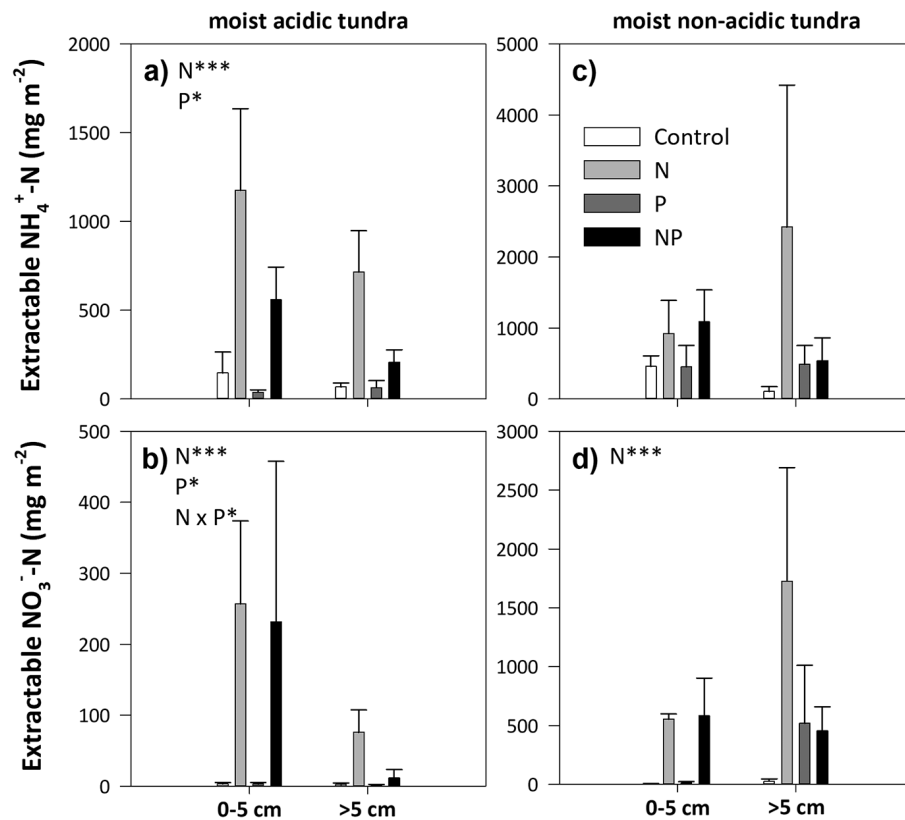


Fig. 5. The effects of long-term nutrient additions on water-extractable soil ammonium-N (a,c), and nitrate-N (b, d), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0–5 cm) and deep organic (>5 cm) soil. The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Asterisks next to text in plots represent significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA ($N \times P \times \text{Depth}$) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

B. nana response, in a Canadian mesic birch tundra, Zamin and Grogan (2012) and Zamin et al. (2014) found that after six to eight years of fertilization both N and P additions independently increased *B. glandulosa* apical stem growth and P alone resulted in an increase in leaf growth. Regardless, this response may still be driven by N, as Zamin and Grogan (2012) attributed the increases in growth with P to an increase in N availability which resulted from P addition. Also in contrast to our findings, in heath tundra in Sweden, Jonasson et al. (1999) found that although *B. nana* was present in the heath plant community, NP fertilization did not lead to *B. nana* dominance and rather increased biomass

across all functional groups, possibly because the fertilization treatment was relatively short (5 yr) in comparison with the experiments we describe here.

The plant functional group abundance we describe in the NP plots after 26 yr of fertilization is largely predicted by shorter-term results from this and other near-by fertilization experiments, yet the plant community appears to be continuing to change. Relatively early in the experiment (four years of fertilization), a decline in moss, graminoids, and forbs and an increase in the abundance of *B. nana* were reported (Chapin et al. 1995, Hobbie et al. 2005). After nine years, moss, graminoids, and evergreen shrubs continued to

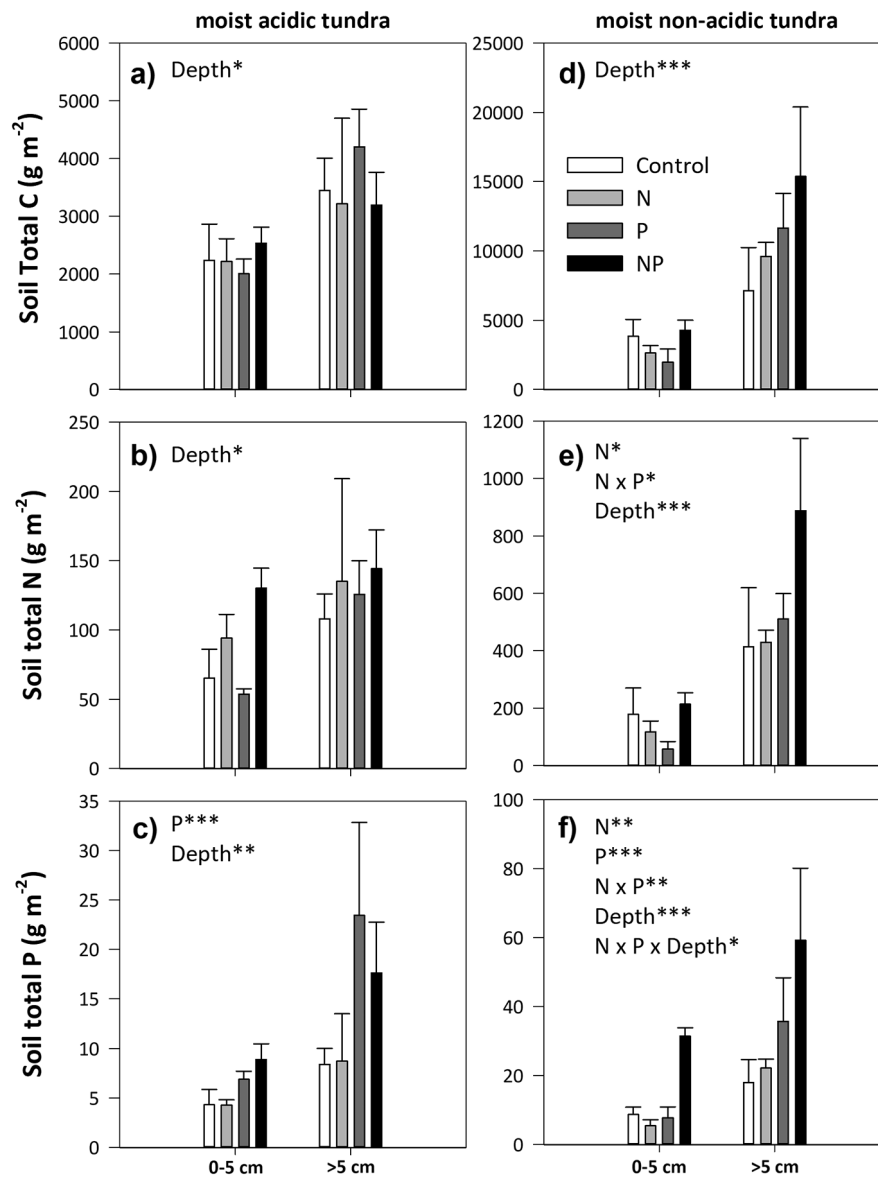


Fig. 6. The effects of long-term nutrient additions on total soil carbon (a,d), nitrogen (b,e), and phosphorus (c,f), in moist acidic tundra (MAT: a,b,c) and moist non-acidic tundra (MNT: d,e,f), each in the shallow (0–5 cm) and deep organic (>5 cm) soil. The treatment Control has received no nutrient additions, N has received 10 g N/yr (as NH_4NO_3), P has received 5 g P/yr (as P_2O_5), and NP has received 10 g N + 5 g P/yr, since 1988 in the MAT and since 1997 in the MNT. Refer to text for further experimental details. Bars are means (MAT, $n = 4$; MNT, $n = 3$) + one standard error. Asterisks next to text in plots represent significant ($\alpha = 0.05$) main effects and interactions from a 3-way factorial ANOVA ($N \times P \times \text{Depth}$) within each ecosystem (degrees of freedom: MAT: 1,21; MNT: 1,14). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

decline (although were still present in the community) and there were continued large increases in dominance by *B. nana* (Chapin et al. 1995, Shaver et al. 2001). After 15 yr of fertilization, there

was strong dominance by *B. nana* (90% of total biomass), with the next most abundant plant (*Rhododendron palustre*, an evergreen shrub) making up only 4% of the biomass, followed by a low

growing perennial forb *Rubus chamaemorus* at <2% biomass (Shaver et al. 2001). Currently, after 26 yr of fertilization, while *B. nana* remains dominant, we also report an increase in the abundance of *R. chamaemorus* with cover reaching nearly 40% in NP plots. Although our characterization of species change is by proportional cover and not biomass, which is more commonly reported from these experiments, there are strong correlations in this ecosystem between cover and biomass (Gough and Hobbie 2003). This increase in *R. chamaemorus* may be facilitated by the recent dieback of *B. nana* in fertilized plots where standing dead *B. nana* makes up 10% of the canopy, removing potential light limitation for the *R. chamaemorus* growing in the understory.

In contrast with the MAT ecosystem, there were very few effects of 16 yr of fertilization by P on vegetation cover in the MNT, supporting our prediction of stronger responses to P addition in the MAT. Further, in contrast with the MAT where responses have become stronger over time, long-term effects on the MNT resemble those on the shorter term. After 4 yr of fertilization, Gough and Hobbie (2003) reported an increase in aboveground biomass with NP addition, resulting primarily from increases in graminoids, and a smaller decrease in biomass with P addition. After 16 yr of treatment, however, we found no effects of fertilization by either nutrient on NDVI-biomass. Gough and Hobbie (2003) also reported increases in graminoid and forb cover and decreases in evergreen shrubs within plots that had been fertilized by both nutrients (but not in plots with single nutrient additions). After 12 more years of fertilization, we found no evidence of this earlier reported co-limitation and the only changes in plant cover we detect are an increase in graminoids. Earlier responses, similar to those we report, were primarily driven by graminoids, and *Carex bigelowii* in particular (Gough and Hobbie 2003). In the MAT, any positive effect of fertilization on graminoids is overwhelmed by the large increases in *B. nana* cover, which quickly outcompetes the other species. *Betula nana* is absent from the MNT and, as predicted by Gough and Hobbie (2003), long-term community changes in this community were subtle because of a lack of species which respond dramatically to fertilization treatments. Further, as suggested by Jonasson et al. (1999), the

successional pathway when nutrient availability increases in different arctic ecosystems is going to depend on both the plant community and soil and microbial properties in that ecosystem, and thus, we must be careful in extrapolating the results to other arctic ecosystems.

Belowground biomass and activity response to multi-decadal nutrient amendments

A release of microbial nutrient limitation may be indicated by an increase in MBC and a decrease in the microbial acquisition effort for that nutrient (i.e., activity of the enzyme[s] that increases the availability of that nutrient), according to threshold element ratio (TER) theory (Sterner and Elser 2002, Sinsabaugh and Follstad Shah 2012). In our study, MBC only responded positively to P addition and only in the deep organic soil of the MAT (Fig. 2a). True to TER theory, this MBC increase with P addition was accompanied by lower phosphatase activity (Fig 3c), which typically indicates a decrease in microbial attempts at P-acquisition (although some of this decrease may be from the phosphate ion competing for the enzyme active site). This match with theory for P amendments has been found in other organic soil systems (Pinsonneault et al. 2016), including these same soils with NP addition in an earlier study (Koyama et al. 2013). In fact, this P-acquisition response to P amendments is more common than C- or N-acquisition responses in the literature (reviewed in Burns et al. 2013), perhaps because the P-acquisition enzyme is more specific (N-acquisition via NAG also acquires C), or because phosphatase production is more universal in microbial communities, as it is a relatively simple trait to acquire (encoded by one gene; Martiny et al. 2015). Combined, the increased biomass and decreased acquisition effort we found provides support for microbial P limitation in these MAT organic soils. In contrast, MNT organic soil and MAT mineral soil microbes appear to be opportunistic about P storage since P addition increased MBP in deep organic soils of the MNT without also increasing MBC (Appendix S1: Table S4). Microbial P storage in tundra soils has been reported before (Chapin et al. 1978, Buckeridge et al. 2016) and slow turnover of P from microbial biomass may provide a mechanism for tundra microbes to overcome permafrost-locked mineral P stores

(Jonasson et al. 1999)—the mineral soils in the MNT were not ice-free in the year that we sampled, for instance. Previous studies have indicated that the MNT system may be less limited by P, despite higher available Ca and Mg concentrations (Whittinghill and Hobbie 2012) which could bind and reduce P availability. For instance, the MNT has higher soil P availability and foliar P concentrations, compared to the MAT (Hobbie and Gough 2002). Our biomass and enzyme results from these long-term nutrient amendments support these earlier conclusions that the MNT is less P-limited than the MAT.

Based on the paradigm that these tundra ecosystems are strongly N-limited, we found surprisingly few effects of independent N additions on soil microbial biomass or activity in either ecosystem. An earlier short-term laboratory incubation of control MAT soil with N amendments concluded microbial N limitation for the MAT (Sistla et al. 2012). However, our long term in situ results found no MBC response to N additions in either ecosystem. These contradictory laboratory and field responses may be due to competition or community differences in laboratory and field. Field microbes may not be accessing the available N, and we believe the most likely reason is the very strong and competitive biomass response in the MAT by *B. nana* (Chapin et al. 1995, Jonasson et al. 1999, Shaver et al. 2001). Alternatively, or in addition, the in situ microbial community may have changed structure (Deslippe et al. 2005) and/or physiology in response to a long-term high N supply. Interestingly, independent additions of N strongly decreased MBN in the MAT deep organic soil (Fig. 2b). MBN declines could be a result of several mechanisms, including direct N (or P) toxicity and associated declines in base cation or C availability (reviewed in Treseder 2008) or declines of Acidobacteria with N (Ramirez et al. 2012), a dominant tundra bacterial phylum (Chu et al. 2010). The lack of decline in MBC with N or P additions implies that long-term fertilizer amendment did not depress total microbial population sizes, but that some other mechanism, such as community restructuring, alters ecosystem N pools by reducing the typically substantial portion of (extractable) soil N that is stored in tundra microbial biomass.

Unlike the microbial biomass response to either N or P independently, most enzymes responded interactively to N and P. An exception to this was the positive response of the cellulose-degrading β -glucosidase to N additions in the MNT. Based on an earlier study in the MAT NP plots, we expected a stimulation of C-degrading enzymes in the MAT system (Koyama et al. 2013). However, our results raise an important caveat of enzyme activity assays: We measured two C-acquiring enzymes (β -glucosidase and NAG) and found that only NAG was stimulated in the shallow MAT NP soils, whereas Koyama et al. (2013) found that NP stimulated most C-degrading enzymes (although, as with this study, not β -glucosidase). Therefore, we suggest caution when using single enzymes as ecosystem indicators; multiple enzymes within each nutrient acquisition group provide a more robust response. Nonetheless, in both ecosystems, we found interactive effects of N and P on N-acquiring and P-acquiring enzyme activity: In the MAT, the reduction in P- or N-acquisition activity with P or N, respectively, is lessened with the addition of the other nutrient (Fig. 3b, c). In the MNT, P and N additions additively increase P- or N-acquisition activity (Fig. 3e, f). These interactive responses in the MNT are likely indicative of the high microbial N and P costs for enzyme fabrication.

Extractable pools provide mixed messages in long-term nutrient addition experiments, as they represent the short-term net sum of nutrients not taken up by plants and microbes (i.e., not limiting) plus the result of any priming of native soil nutrient pools (i.e., limiting), in a temporally unstable pool. Nonetheless, broad conclusions can be drawn from these data. Not surprisingly, in both systems available N pools typically increased with N amendments and available P with P amendments. However, available N and P pools were generally lower when added together in the MAT, implying short-term co-limitation of N and P in that system, in particular with regard to nitrate availability. Similar interactions were found in a 7-yr nutrient amendment study in the Canadian low arctic tundra, although in that case ammonium availability was particularly sensitive to NP interactions and declined when P was added simultaneously (Zamin et al. 2014).

Belowground total soil C, N, and P response to multi-decadal nutrient amendments

Soil total C stocks provide a long-term signal of the effects of N and P on SOM decomposition, and SOM stabilization processes, and thus summarize above- and belowground long-term ecosystem response to N and P fertilization. Surprisingly, we found a lack of effects of N or P on soil total C in the MAT, unlike reported C-losses in the same system, after shorter periods of nutrient amendment (5 yr, Koyama et al. 2013; 6 yr, Nadelhoffer et al. 2002; and 19 yr, Mack et al. 2004). However, our results parallel other long-term nutrient amendment results in the same system (22 yr of amendments; Koyama et al. 2013), implying that short-term ecosystem C-loss with the removal of plant–nutrient limitations does not persist beyond two decades. This is possibly a response to aboveground community shifts over time, as summarized above: Earlier soil total C-loss corresponded with increases in deciduous shrub biomass (Mack et al. 2004), which has now started to decline.

Soil total P accumulates with P additions in the MAT. In contrast to this independent effect of P in the MAT, in the MNT system we found interactive effects of N and P (and depth). We saw an increase in N and P storage with NP amendment in the organic soils in addition to much higher enzyme activity in this ecosystem (compare ecosystem axes in Fig. 3) and in the NP plots in particular. This higher enzymatic activity is in contrast with lower microbial respiration in the MNT ecosystem (Whittinghill and Hobbie 2012) and may imply higher carbon use efficiency, at least when nutrient limitations are removed.

We suggest that the strong interactive effects belowground in the MNT reflect the low fertilization response aboveground in this system. In the MAT, belowground responses may be preempted by strong competition by *B. nana* in particular for both N and P and as a result we see repressed enzyme activity and low microbial biomass. In contrast, the MNT vegetation community contains fewer long-term responsive plant species and is associated with belowground enhanced enzyme activity and available nutrients. It is possible that this has promoted much larger microbial biomass and total C, N, and P stores in the MNT (compare ecosystem y-axes in Figs. 2 and 6), especially in the NP plots.

In summary, whereas the MAT ecosystem was dominated by an aboveground response, especially *B. nana*, to NP, the MNT ecosystem was dominated by the belowground response (Table 2). Effects of fertilization on ecosystems are often characterized based on the response aboveground, even though the belowground response may differ or may even respond in the absence of an aboveground effect (Jonasson et al. 1999). Two clear conclusions from these very different above- and belowground and ecosystem trajectories are (1) arctic ecosystems are not universally N-limited but also exhibit complex responses to P alone or in combination with N; and (2) the presence or absence of key vegetation species (*B. nana* and *R. chamaemorus*) can cascade from aboveground to belowground and restrict the extrapolation of responses of nutrient addition in a single arctic ecosystem to other arctic ecosystems, the short term to the long term, or aboveground to belowground. Therefore, we cannot generalize the response of arctic tundra ecosystems to long-term nutrient amendment. An extension of this conclusion is that we should use caution before assuming that arctic ecosystems will respond similarly to a changing world, and should broaden our long-term manipulations to multiple different tundra ecosystems, with different vegetation and microbial communities, different C stocks, and potentially different sensitivities to global change.

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DATA ACCESSIBILITY

Species cover: Arctic Data Center Entry <https://doi.org/10.6073/pasta/8a2999c9ed297a184aaca7057e1ae177>. Soil microbial biomass C, N and P; extracellular enzyme activity, soil extractable C, N and P; soil total C, N, and P, in g/m² and µg/g¹: Arctic Data Center Entry <https://doi.org/10.6073/pasta/2302b3a5eab56970aa4e4f71d36b7fce>.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2735/full>